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***AKT1* polymorphisms are associated with risk for metabolic syndrome**

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Abstract Converging lines of evidence suggest that AKT1 is a major mediator of the responses to insulin, insulin-like growth factor 1 (IGF1), and glucose. AKT1 also plays a key role in the regulation of both muscle cell hypertrophy and atrophy. We hypothesized that *AKT1* variants may play a role in the endophenotypes that make up metabolic syndrome. We studied a 12-kb region including the first exon of the *AKT1* gene for association

with metabolic syndrome-related phenotypes in four study populations [FAMUSS cohort ($n = 574$; age 23.7 ± 5.7 years), Strong Heart Study (SHS) ($n = 2,134$; age 55.5 ± 7.9 years), Dynamics of Health, Aging and Body Composition (Health ABC) ($n = 3,075$; age 73.6 ± 2.9 years), and Studies of a Targeted Risk Reduction Intervention through Defined Exercise (STRRIDE) ($n = 175$; age 40–65 years)]. We identified a three SNP haplotype that we call H1, which represents the ancestral alleles at the three loci and H2, which represents the derived alleles at the three loci. In young adult European Americans (FAMUSS), H1 was associated with higher

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fasting glucose levels in females. In middle age Native Americans (SHS), H1 carriers showed higher fasting insulin and HOMA in males, and higher BMI in females. In older African-American and European American subjects (Health ABC) H1 carriers showed a higher incidence of metabolic syndrome. Homozygotes for the H1 haplotype showed about twice the risk of metabolic syndrome in both males and females ($p < 0.001$). In middle-aged European Americans with insulin resistance (STRIDE) studied by intravenous glucose tolerance test (IVGTT), H1 carriers showed increased insulin resistance due to the Sg component ($p = 0.021$). The 12-kb haplotype is a risk factor for metabolic syndrome and insulin resistance that needs to be explored in further populations.

Introduction

AKT1 [also called protein kinase B (PKB)] is involved in two major signaling pathways: the regulation of muscle hypertrophy and atrophy (Nader 2005) and AKT1 is mediator of the biological functions of insulin and augments glucose uptake in muscle, adipocytes and liver along with a host of other tissues (Elghazi et al. 2006; Zdychova and Komers 2005). AKT1 mediates glucose uptake by inducing translocation of vesicles containing GLUT4 from intracellular stores to the plasma membrane (Hajduch et al. 1998). Briefly, upon insulin stimulation, AKT1 agonists activate receptor kinase functions that result in the stimulation of tyrosine phosphorylation of substrates such as IRS1 (Sun et al. 1991) and IRS2 (Sun et al. 1995). Tyrosyl phosphorylation of the IRSs provides binding sites for specific proteins containing Src-homology 2 (SH₂) domains such as PI3K. PI3K is responsible for the insulin

stimulation of GLUT4, the major insulin-regulated glucose transporter from intracellular vesicles to the plasma membrane in insulin-sensitive cells (Frevet et al. 1998).

In order to look at the function of AKT1 in pancreatic beta cells, researchers over-expressed constitutively active AKT1 in the β -cells of mice resulting in augmented β -cell mass by an increase in proliferation and cell size (Bernal-Mizrachi et al. 2001; Tuttle et al. 2001), and resistance to streptozotocin-induced diabetes (Bernal-Mizrachi et al. 2001; Tuttle et al. 2001). Mice with reduced Akt/Pkb activity in β -cells exhibited normal β -cell mass but defective insulin secretion (Bernal-Mizrachi et al. 2004), and increased susceptibility to developing glucose intolerance and diabetes following fat feeding (Bernal-Mizrachi et al. 2004). The roles of AKT1 as a key mediator in both muscle hypertrophy and atrophy and insulin signaling and glucose uptake indicate that this gene may play a role in a metabolic phenotypes, specifically the endophenotypes that together lead to metabolic syndrome.

Thus, we studied the *AKT1* gene as a candidate for metabolic syndrome features. We hypothesized that the use of a young adult population would increase sensitivity for detection of these phenotypes, as all are well-documented to be age-related. Further, each phenotype has many potential environmental influences (lifestyle, training, diet), thus we hypothesize that a college-age population might have fewer potential confounders (Thompson et al. 2004; Uthurralt et al. 2007; Zoeller et al. 2007).

Here, we report the *AKT1* haplotype structure, including a relatively small region of 12 kb with two predominant haplotypes (H1 and H2) upstream and inclusive of the first exon of the *AKT1* gene. We evaluated a hap-tag SNP (rs1130214) in three population-based cohorts (FAMUSS, SHS, Health ABC), and one cohort focused on insulin resistance and response to training (STRIDE) (Harris et al. 2005; Lee et al. 1990; Slentz et al. 2007; Thompson et al. 2004). We show that the H1 12 kb *AKT1* haplotype is significantly associated with higher fasting serum glucose levels, and an increased risk for developing metabolic syndrome.

Participants and methods

Subject populations

FAMUSS population

The study design of the FAMUSS Study has been reported (Thompson et al. 2004). Briefly, 574 volunteers (18–41 years; mean 23.7 ± 5.7) were enrolled into a unilateral supervised 12-week resistance training intervention of the non-dominant arm. Quantified phenotypes included

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baseline blood pressure, height and weight and serum measurements including HDL, total cholesterol, fasting glucose, fasting insulin, and triglycerides. A dichotomous characterization of metabolic syndrome was defined as fulfilling at least three of the five criteria proposed by NCEP (2001). These criteria include an elevated waist circumference, triglycerides, blood pressure, fasting glucose and reduced HDL cholesterol. Because the FAMUSS Study did not collect waist circumference measurements, an elevated BMI (>25) was used as a surrogate.

Strong Heart Study

The SHS is a study of CVD and its risk factors among American Indian men and women (Lee et al. 1990). Briefly, DNA and serum measurements were collected on 2,134 volunteers (45–75 years; mean 55.5 ± 7.9). Quantified phenotypes included baseline blood pressure, height and weight and serum measurements including HDL, total cholesterol, fasting glucose, fasting insulin, triglycerides and incidence of metabolic syndrome.

Health ABC Study

Health ABC is a longitudinal study of body composition in older men and women with goals of identifying the clinical conditions that accelerate changes in and examining the health impact of these changes on the diseases of old age (Visser et al. 1999). Briefly, DNA and serum measurements were collected on 3,075 volunteers (68–80 years; mean 73.6 ± 2.9). Quantified phenotypes included baseline blood pressure, height and weight and serum measurements including HDL, total cholesterol, fasting glucose and fasting insulin. Triglycerides and incidence of metabolic syndrome and diabetes were classified as dichotomous phenotypes using NCEP ATP III criteria.

STRRIDE Study

The STRRIDE Study is a controlled study designed to measure the effect of exercise on abdominal fat. Briefly, 175 overweight individuals, male and female, aged 40–65 years were assigned an 8-month exercise regimen. All participants had mild to moderate lipid abnormalities. They were randomly assigned to one of three exercise groups: (1) high amount/vigorous intensity (65–80% of peak oxygen consumption), (2) low amount/vigorous intensity (65–80% of peak oxygen consumption), and (3) low amount/moderate intensity (40–55% of peak oxygen consumption). Quantified phenotypes included tomography scans of abdominal fat, BMI measures, Sg (glucose-stimulated glucose-release), insulin sensitivity index (ISI) and acute insulin response to glucose (AIRg).

Phenotype measures for FAMUSS Study

Serum measurements were made from stored fasting sera and plasma from each subject (baseline). Measurements: fasting glucose, fasting insulin, C-reactive protein and lipid panel (triglycerides and HDL). All measurements were made by Quest Diagnostics (Chantilly VA).

SNP discovery

SNPs for each exon, exon/intron boundaries, and 10 kb upstream (5') to the *AKT1* gene were identified by screening a panel of 96 ethnically diverse genomic DNA samples with denaturing high performance liquid chromatography (Transgenomic WAVE system, Transgenomic, USA) and automated sequencing (Applied Biosystems 3100 Genetic Analyzer). Polymorphisms were tested for allele frequency, and cross-referenced to dbSNP (<http://www.dbSNP.org>) and HapMap (<http://www.hapmap.org>) data resources. Twelve SNPs were studied in depth, selected based upon allele frequency and linkage disequilibrium testing. The published sensitivity of DHPLC for SNP detection is 96% (Jones et al. 1999). However, to reach that level of SNP detection requires optimal temperature selection and possibly primer redesign (Jones et al. 1999). For this publication, we cannot be certain that we did not miss additional variation in the *AKT1* regions that we scanned due to the level of sensitivity of this technique. In addition, DHPLC suffers from the inability to discover homozygote changes and would require mixing of a sample with a known sequence to all scanned samples for the discovery of homozygous changes (Mogensen et al. 2003). DHPLC Novel SNPs not in dbSNP or HapMap databases were submitted to dbSNP. A list of the SNPs studied and their respective dbSNP identifiers (including those submitted to dbSNP) can be found in Table 1.

Genotyping

Genotyping was done using a novel TaqMan allele discrimination assay that employs the 5' nuclease activity of Taq polymerase to detect a fluorescent reporter signal generated during PCR reactions (see Supplemental Table 1). Both alleles were detected simultaneously using allele-specific oligonucleotides labeled with different fluorophores, and genotypes determined by the ratio of the two fluorophores used. Allele-specific PCR reactions for each SNP included 10 ng genomic DNA, 900 nM forward and reverse PCR primers, 200 nM fluorescent allele discrimination probes (common allele FAM labeled; rare allele VIC labeled) and TaqMan® Universal PCR Master Mix, No AmpErase® UNG [Applied Biosystems (ABI), Foster City, CA, USA] in a final volume of 10 μ l. The PCR

Table 1 *AKT1* single nucleotide polymorphisms discovered using DHPLC in a 96-individual panel

SNP position in <i>AKT1</i> ^a	SNP rs#	
–C9756A	rs33925946	Novel
–C6148T	rs4983387	
–C6024T	rs10141867	
–C5854T	rs34284721	Novel
–C832G	rs2494750	
+G1780A	rs28634999	Novel
+G2347T	rs1130214	
+G2375A	rs10138227	
+A15756T	rs2494737	
+G20703A	rs3730346	
+A22889G	rs2494732	
+G23477A	rs2498800	

^a Numbering based on Genbank NM_001014431.1

and fluorescent ratio profile was done using 10 min at 95°C (denaturation), and 44 cycles of 15 s at 92°C and 1 min at an annealing temperature of 60°C. Reactions were set up using a MWG robot, and fluorescence ratios and allele calling done using an ABI 7900.

Linkage disequilibrium

Pair-wise tests for linkage disequilibrium were done for all 12 *AKT1* polymorphisms using the correlation coefficient (*R*) on each pair-wise combination in the 1,078 FAMUSS subjects. A haplotype minimization algorithm contained within the PHASE v 2.1 program (Stephens et al. 2001) was then used to establish haplotypes from these data.

Statistical methods

Hardy–Weinberg equilibrium was determined for each SNP using a χ^2 test to compare the observed genotype frequencies to those expected under H–W equilibrium. All genotyped SNPs were in H–W equilibrium.

All metabolic syndrome-related phenotypes were analyzed as either continuous quantitative traits (triglycerides, fasting glucose, etc.) or as dichotomous traits (metabolic syndrome and high/low triglyceride level). Normality of each quantitative trait was confirmed using the Shapiro–Wilk normality test.

Bivariate correlation analyses of each quantitative measurement showed several significant correlations with age and baseline mass; therefore, associations between each SNP and quantitative phenotype were assessed using analysis of covariance (ANCOVA) methods. Due to large gender differences all analyses were performed separately for males and females.

Each ANCOVA used a dominant genetic model statistically comparing the group of homozygous wild-type individuals to the group of heterozygous and homozygous mutant individuals.

Linear regression analysis, including likelihood ratio tests between full (containing genotype and covariates) and constrained (containing covariates only) models, were performed to estimate the proportion of variance in volumetric measurements attributable to each SNP's genotype. For dichotomous traits, logistic regression models adjusted for age and baseline body mass were used. A nominal *p* value of 0.05 was defined as significant and all statistical analyses were performed using Stata V.8 (StataCorp, College Park, TX).

Results

SNP discovery and haplotypes of *AKT1*

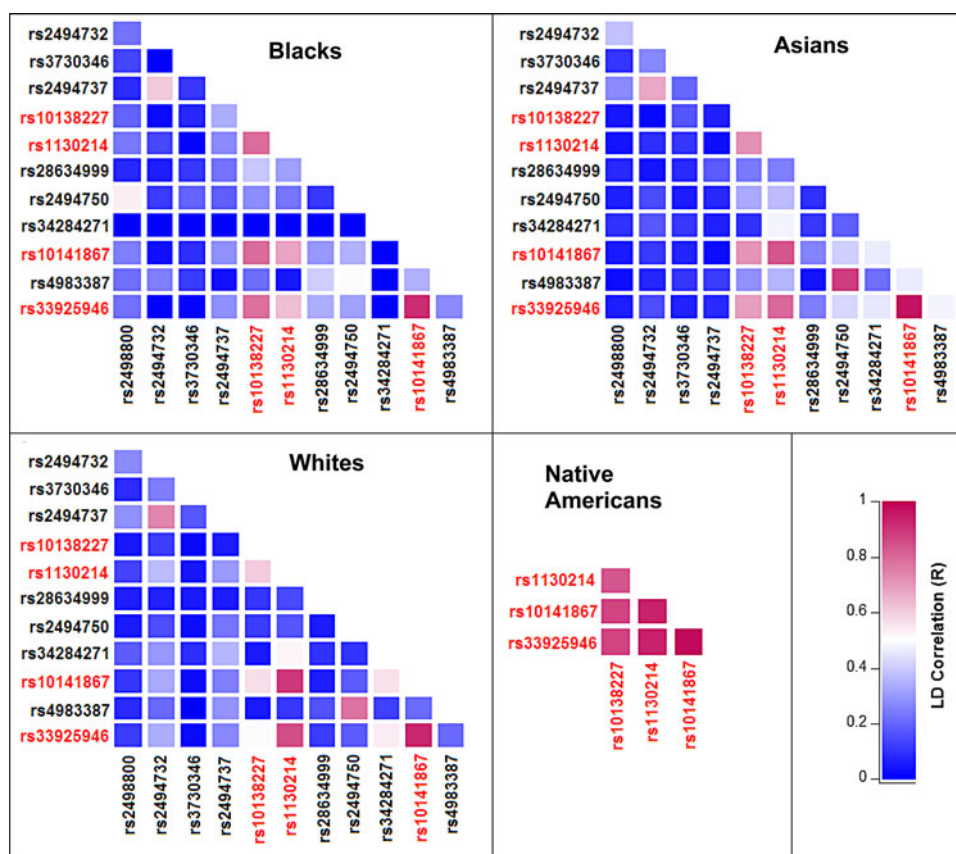
SNPs for each exon, exon/intron boundaries, and 10 kb upstream (5') to the *AKT1* gene (Genbank NM_001014431.1) were identified by screening a panel of 96 ethnically diverse genomic DNA samples with denaturing high performance liquid chromatography and automated sequencing. We identified 12 SNPs in *AKT1*, 7 of which were intragenic and 5 of which were upstream of the gene. A list of the SNPs studied and their respective dbSNP identifiers (including three novel SNPs submitted to dbSNP) can be found in Table 1.

The SNPs were genotyped in the FAMUSS population (Table 2) to identify regions that showed strong linkage disequilibrium to test in additional populations. Two major haplotype blocks were identified, one including most of the coding region of the gene and one 12 kb haplotype upstream of *AKT1* inclusive of the first exon, haplotype H1/H2. The H1/H2 haplotype is composed of SNPs: rs1130214, rs10141867 and rs33925946. The H1 haplotype alleles are GCC for those three SNPs; the H2 haplotype alleles are TTA for those three SNPs (on the plus strand DNA). LD plots were generated showing the extent of LD between the SNPs listed in Table 1. The SNPs of haplotype H1/H2 are shown in red text. The amount of LD between

Table 2 Characteristics of the FAMUSS cohort

Characteristic	Females		Males	
	<i>N</i>	Mean \pm SD	<i>N</i>	Mean \pm SD
Age (years)	359	23.58 \pm 5.62	215	24.29 \pm 5.90
Weight (kg)	359	65.3 \pm 12.8	215	81.2 \pm 17.2
Height (cm)	359	165 \pm 6.8	215	177.8 \pm 6.8
BMI	359	23.94 \pm 4.66	215	25.61 \pm 5.15

Fig. 1 Linkage disequilibrium among *AKT1* SNPs within the FAMUSS population and SHS. Linkage disequilibrium is plotted for African-Americans, Asians and European Americans of the FMS cohort. The LD was calculated between each pair of SNPs using the r^2 measurement and haplotype frequencies were estimated using the online program PHASE, which uses genotype data to determine the phase of the chromosomes and thus haplotype structure and frequency. The SNPs from Table 1 are plotted on the *X* and *Y* axes. A color scale is then used to plot the LD between any two SNPs. Blue equals an r^2 of zero, red equals an r^2 of 1.0. The SNPs of H1/H2 are in red text. All three of the SNPs are in high LD with each other as shown by the red blocks



any two SNPs (plotted on the *X* and *Y* axis) as determined by the correlation coefficient R is shown on a color scale from blue to red. Blue equals r^2 of zero, or no LD; red equals r^2 of 1 or perfect LD (Fig. 1). SNP rs10138227 shows some LD with the other three but it is not strong enough to include as part of the haplotype. From this work, we chose to analyze rs1130214 as a hap-tag SNP representing a 12 kb haplotype covering the first intron/10 kb upstream.

Associations between *AKT1* and the FAMUSS, Strong Heart Study, Health ABC and STRRIDE studies

We used the young adult FAMUSS population to screen for associations between the *AKT1* gene polymorphisms of the H1/H2 haplotype and metabolic syndrome endophenotypes: increased adiposity, increased blood pressure, low serum HDL, insulin resistance and fasting glucose levels, with subsequent validation of associations in additional population-based cohorts (SHS, Health ABC) and in the insulin resistant population STRRIDE (demographics of these four populations are shown in Tables 2, 3, 4, 5).

The SNP association analyses were done with both heterozygotes (GT) and homozygotes for the T allele (TT) in the same group because the number of TT individuals was too low in some groups to see an effect. Therefore, the analyses

Table 3 Characteristics of the Strong Heart cohort

Characteristic	Females (<i>N</i> = 1,349)	Males (<i>N</i> = 785)
Tribe		
Arizona	724	357
South Dakota	625	428
Age (years)	56.19 ± 7.87	55.45 ± 7.66
Weight (kg)	81.11 ± 17.10	89.37 ± 19.33
Height (cm)	159.47 ± 5.66	173.14 ± 6.35
BMI	31.89 ± 6.52	29.78 ± 6.07

are interpreted as associations with at least one copy of the T allele (H2) versus homozygotes for the G allele (H1).

The H1/H2 hap-tag SNP rs1130214 was associated with fasting glucose only in females and this SNP contributed 1.3% to genotypic variance (Table 6). SNP rs1130214 causes a G to T change (representing the H1 and H2 haplotypes, respectively). With at least one copy of the T allele, females had lower values of fasting glucose (GG, *N* = 201; 86.22 ± 0.50 mg/dL vs. GT/TT, *N* = 178; 84.55 ± 0.53 mg/dL; *p* = 0.0236). This SNP did not have any associations with any metabolic syndrome endophenotypes in males in the FAMUSS Study.

We then used rs1130214 as a hap-tag SNP, and genotyped both Strong Heart Study (Table 3) and Health ABC (Table 4)

Table 4 Characteristics of the Health ABC cohort

Characteristic	African-Americans		Caucasians	
	Females (<i>N</i> = 729)	Males (<i>N</i> = 552)	Females (<i>N</i> = 855)	Males (<i>n</i> = 939)
Site				
Memphis	337	276	459	476
Pittsburgh	392	276	396	463
Age (years)	73.37 ± 2.95	73.51 ± 2.78	73.60 ± 2.79	73.92 ± 2.92
Weight (kg)	75.60 ± 15.76	81.44 ± 14.73	66.25 ± 12.21	81.36 ± 12.38
BMI	29.66 ± 5.86	27.18 ± 4.40	26.03 ± 4.54	27.00 ± 3.68

Table 5 Characteristics of the STRIDDE cohort

Characteristic	African-Americans		Caucasians	
	Females (<i>N</i> = 52)	Males (<i>N</i> = 32)	Females (<i>N</i> = 139)	Males (<i>n</i> = 154)
Age (years)	50.17 ± 4.66	49.03 ± 6.18	54.93 ± 5.14	50.44 ± 7.04
Weight (kg)	85.89 ± 12.67	96.74 ± 13.67	78.34 ± 10.75	95.68 ± 12.28
Height (cm)	163.39 ± 6.83	176/02 ± 7.98	163.22 ± 6.19	177.95 ± 5.83
BMI	32.07 ± 3.46	31.20 ± 3.73	29.36 ± 3.25	30.14 ± 2.91

Table 6 Association of the T allele of rs1130214 in FAMUSS with decreased glucose

SNP	Phenotype	Gender	<i>N</i> , adjusted mean ± SEM	<i>p</i> value	% variation attributable to genotype
rs1130214	Fasting glucose	Female	GG (<i>N</i> = 201; 86.22 ± 0.50) GT/TT (<i>N</i> = 178; 84.55 ± 0.53)	0.0236	1.3%

to test for any associations with metabolic phenotypes in older cohorts. For the older and more heterogeneous Strong Heart Study population of Native Americans, rs1130214 showed association with 2-h glucose, fasting insulin, triglycerides, and the HOMA measure of insulin resistance in males (Table 7). The T allele was associated with lower 2 h glucose levels (GG, *N* = 434; 180.69 ± 5.11 mg/dL vs. GT/TT, *N* = 147; 160.01 ± 8.77 mg/dL; *p* = 0.0422) and lower fasting insulin (GG, *N* = 564; 19.44 ± 0.68 μIU/mL vs. GT/TT, *N* = 208; 16.21 ± 1.12 μIU/mL; *p* = 0.0136). In addition, in males, the T allele was associated with a lower HOMA value (GG, *N* = 559; 7.52 ± 0.32 vs. GT/TT, *N* = 203; 6.15 ± 0.53; *p* = 0.267).

In male participants in the Health ABC Study, the T allele of rs1130214 was associated with lower fasting glucose (GG, *N* = 661; 110.5 ± 1.4 mg/dL vs. GT/TT, *N* = 798; 106.4 ± 1.3 mg/dL; *p* = 0.0305) (Table 8). This effect was also seen in African-Americans males (GG, *N* = 205; 116.8 ± 2.9 mg/dL vs. GT/TT, *N* = 328; 107.7 ± 2.3 mg/dL; *p* = 0.0170). These results are not significant after correction for multiple testing (*p* < 0.007); however, the statistical community is divided on correction methods for multiple testing since the Bonferonni correction may be too harsh and thus increasing false negatives (Rice et al. 2008).

Taken together, associations from all three population-based cohorts suggested that the *AKT1* upstream haplotype was a determinant of insulin resistance, resulting in higher circulating glucose and/or insulin with apparent age- and sex-dependence.

Associations between *AKT1* rs1130214 and the Health ABC Study for metabolic syndrome

The five commonly accepted endophenotypes of metabolic syndrome are: increased insulin resistance or fasting glucose plasma levels (>100 mg/dl), increased adiposity (central obesity or BMI > 30), elevated triglycerides (equal to or greater than 150 mg/dL), increased blood pressure (>130mmHg/85mmHg), and low serum HDL (<40–50 mg/dl) (Alberti et al. 2006; Alberti and Zimmet 1998; Grundy et al. 2004; NCEP 2001). To determine if the H1/H2 haplotype is a major determinant of metabolic syndrome diagnosis, we studied the Health ABC population. This population-based study had a diagnosis of T2D as an exclusion criterion, with excellent phenotyping of metabolic syndrome endophenotypes in a tightly restricted age range (Harris et al. 2005).

The incidence of metabolic syndrome showed a significant association with rs1130214 in the Health ABC

Table 7 Association of the T allele of rs1130214 in SHS with parameters of metabolic syndrome

SNP	Phenotype	Gender	N, adjusted mean \pm SEM	<i>p</i> value	% variation attributable to genotype
rs1130214	BMI	Female	GG (<i>N</i> = 1,131; 31.87 \pm 0.19) GT/TT (<i>N</i> = 396; 31.04 \pm 0.32)	0.0271	0.3
rs1130214	2 h glucose	Male	GG (<i>N</i> = 434; 180.69 \pm 5.11) GT/TT (<i>N</i> = 147; 160.01 \pm 8.77)	0.0422	0.7
rs1130214	Fasting insulin	Male	GG (<i>N</i> = 564; 19.44 \pm 0.68) GT/TT (<i>N</i> = 208; 16.21 \pm 1.12)	0.0136	0.6
rs1130214	Triglycerides	Male	GG (<i>N</i> = 565; 142.66 \pm 9.92) GT/TT (<i>N</i> = 208; 189.74 \pm 16.35)	0.0141	0.8
rs1130214	HOMA	Male	GG (<i>N</i> = 559; 7.52 \pm 0.32) GT/TT (<i>N</i> = 203; 6.15 \pm 0.53)	0.0267	0.6

Table 8 Association of the T allele of rs1130214 in HABC cohort with decreased fasting glucose

SNP	Phenotype	Gender/Ethnicity	N, Adjusted mean \pm SEM	<i>p</i> value	% variation attributable to genotype
rs1130214	Fasting glucose	All males	GG (<i>N</i> = 661; 110.5 \pm 1.4)* GT/TT (<i>N</i> = 798; 106.4 \pm 1.3)*	* <i>p</i> = 0.0305	0.3
rs1130214	Fasting glucose	African-American males	GG (<i>N</i> = 205; 116.8 \pm 2.9)* GT/TT (<i>N</i> = 328; 107.7 \pm 2.3)*	* <i>p</i> = 0.0170	1.1

Table 9 The T allele of rs1130214 is protective of metabolic syndrome in HABC cohort

Group	rs1130214 genotype	N (controls)	N (metabolic syndrome)	Odds ratio	<i>p</i> value	95% confidence interval
All subjects	GG	812	566	1.00		
	GT/TT	1,028	586	0.765	0.001	0.655–0.894
All males	GG	408	251	1.00		
	GT/TT	522	244	0.650	<0.001	0.512–0.826
Caucasian males	GG	275	179	1.00		
	GT/TT	300	169	0.849	0.050	0.558–1.000
African-American males	GG	133	72	1.00		
	GT/TT	252	75	0.545	0.005	0.357–0.834
All females	GG	404	325	1.00		
	GT/TT	476	342	0.854	0.151	0.689–1.059
Caucasian females	GG	236	186	1.00		
	GT/TT	244	167	0.804	0.148	0.598–1.080
African-American females	GG	168	129	1.00		
	GT/TT	232	175	0.978	0.894	0.712–1.346

population (Table 9). The analysis was done for both heterozygotes (GT) and homozygotes for the T allele (TT) because the number of TT individuals was too low for some groups to determine any effect. We saw strong effects with having at least one copy of the T allele suggesting that haplotype H2 strongly influences these phenotypes even in the presence of one copy of the H1 haplotype (represented by the G allele). Individuals with at least one copy of the T allele were much less likely to have metabolic syndrome,

consistent with the lower insulin resistance observed with the T allele (H2) in all three cohorts. This was true for the entire Health ABC cohort (GT/TT: OR = 0.765, *p* = 0.001) and all males (GT/TT: OR = 0.650, *p* < 0.001). There was a significant effect in all females for the TT individuals only (TT, OR = 0.577; *p* = 0.005). The T allele showed a very strong protective effect in African-American males (GT, OR = 0.539; *p* = 0.007). These results are significant after correction for multiple testing (*p* < 0.007).

Table 10 The T allele of rs1130214 was associated with higher Sg in the STRRIDE cohort

Phenotype	Cohort	F test	p value
Baseline Sg	Caucasian males	4.06	0.021*
	African-American males	1.01	NS
	Caucasian females	1.28	NS
	African-American females	2.97	NS

Associations between rs1130214 and the STRRIDE Study for glucose-stimulated glucose-release (Sg) levels

The STRRIDE Study is a cohort of insulin-resistant middle-aged adults. The H2 haplotype (the T allele of rs1130214) was associated with higher Sg in European American males (TT, $p = 0.021$) (Table 10). This is consistent with the other cohorts and H2 associations with insulin resistance phenotypes and even protection against metabolic syndrome, which often follows the development of insulin resistance. There were no significant associations (denoted as NS) with any other group of this cohort (Table 10).

Discussion

We defined the haplotype structure of the *AKT1* gene and studied 12 representative SNPs in a young adult population to identify genetic predispositions to endophenotypes of metabolic syndrome. We identified a relatively small 12 kb haplotype, defined by the SNPs rs1130214, rs10141867 and rs33925946, which are in very strong linkage disequilibrium in all populations studied. In the young adult population (FAMUSS Study), the ancestral haplotype of the 12 kb region (H1; tagged by rs1130214) was associated with higher fasting glucose in females (average age 24 years), suggesting that the 12 kb haplotype is associated with insulin resistance. We validated this finding first in an older and more heterogeneous Native American population (SHS; average age 56 years), where H1 had higher fasting serum glucose, higher serum insulin, and a higher HOMA value in males, all consistent with association with insulin resistance. Although we originally identified an association with fasting glucose levels in the FAMUSS females, we did not see this same association in the older females of the SHS or Health ABC. We then studied a cohort of aged African-American and European American (Health ABC; average age 73 years), and also found evidence for association with insulin resistance, where men with H1 showed higher fasting glucose.

Insulin resistance is one of the five key endophenotypes associated with T2D, and the precursor state of metabolic

syndrome. The diagnosis of metabolic syndrome is made when a subject shows increased visceral adiposity, and two additional threshold phenotypes [increased insulin resistance, increased blood pressure, increased triglycerides (TGs), and low HDL serum levels]. Insulin resistance is a complex physiological and biochemical phenotype involving responses to increased serum glucose by the pancreas, muscle, and liver. The individual components of pancreas, muscle and liver can be resolved by an IVGTT. We studied the association of the 12 kb upstream haplotype with the IVGTT data from STRRIDE subjects with features of metabolic syndrome, and found evidence for association with the Sg phenotype. Thus, four distinct cohorts all validated association of the upstream *AKT1* 12 kb haplotype (H1) with increased insulin resistance; H2 associates with decreased insulin resistance.

Given the association of the 12 kb *AKT1* upstream haplotype with measures of insulin resistance in four cohorts, we queried the association of the same haplotype with metabolic syndrome diagnosis. It is important to note that the H1 haplotype is the more common haplotype in all populations, and thus associations of a common haplotype with a rare trait become more challenging statistically. Of the four populations studied, only one was felt to be adequate for testing of metabolic syndrome associations, namely the Health ABC Study. The FAMUSS Study population was too young to have an appreciable incidence of metabolic syndrome. The Strong Heart Study was quite heterogeneous in age and showed a very high incidence of many comorbidities associated with metabolic syndrome. The STRRIDE Study was a non-population based study, where participants were recruited based upon having features of metabolic syndrome. However, the Health ABC Study was of large size ($n = 3,000$), with tightly restricted age (73.6 years), with metabolic syndrome as a key focus of the study. Genotyping of the upstream haplotype in Health ABC showed a very strong association with metabolic syndrome diagnosis (Table 9). Homozygotes for the ancestral H1 haplotype (GG) had double the risk of metabolic syndrome compared to those homozygous for the less common H2 haplotype (TT). This association was retained when the population was stratified by sex and ethnicity.

Along with protection from metabolic syndrome, the *AKT1* H2 haplotype was associated with higher values of TGs in males in the SHS. This result is counterintuitive to the association of lower 2-h glucose, lower fasting insulin, and lower HOMA value. A current issue in genetics is the extension of associated loci discovered using genome-wide association studies (GWAS) in European ancestry to non-European individuals (Murray et al. 2010; Teslovich et al. 2010). For example, a recent study (Murray et al. 2010) examined SNPs for asthma risk in two African ancestry

groups that were located near a gene encoding *ORMDL3* (17q21) that was identified for asthma risk in individuals with European-descent (Moffatt et al. 2007). The SNPs near the *ORMDL3* gene did not replicate risk for asthma in the African ancestry groups (Murray et al. 2010). Concurrently, another study focusing on the genetics of blood lipids found 24 of 27 SNPs replicated for TG values between European-descent and South Asians, a replication of 26 of 28 SNPs for TG values in East Asians, and a replication of 24 of 30 SNPs for TG values in African-Americans (Teslovich et al. 2010). These results speak of the difficulty that exists in understanding genetic variation and its relationship to different phenotypes in different ethnic groups. The higher TG values associated with H2 haplotype in the SHS may be due an interaction with the lack of access to medications that can control lipids (Stern 1998) and genotype. Finally, there is an ongoing unfavorable increase in the prevalence of hypertension, low HDL, and diabetes mellitus in the aging survivors of the SHS and this will likely lead to further increases in CVD morbidity and mortality. This interaction between *AKT1* H2 and TG will need to be further explored in additional populations.

To our knowledge, the 12 kb haplotype described here is one of the strongest candidate gene studies for either insulin resistance or a metabolic syndrome diagnosis identified to date. In another report, we report function for all the SNPs in the associated haplotype (Harmon et al. 2010, submitted). In future studies, we will examine how this *AKT1* haplotype impacts the effect on insulin resistance when combined with the numerous reported genome-wide association studies (GWAS) of isolated insulin resistance (Lyssenko et al. 2009), insulin response (Rich et al. 2009), fasting glucose (Bouatia-Naji et al. 2008; Dupuis et al. 2010; Prokopenko et al. 2009), glucose response (Saxena et al. 2010), and GWAS studies of the fully morbid T2D diagnosis (Wellcome Trust Case Control Consortium 2007; Rung et al. 2009; Saxena et al. 2007; Scott et al. 2007; Zeggini et al. 2008).

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